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Persistent organochlorine pollutants in human serum of 50–65 years old women in the Flanders Environmental and Health Study (FLEHS). Part 1: concentrations and regional differences

G. Koppen [a], A. Covaci [b,*], R. Van Cleuvenbergen [a], P. Schepens [b], G. Winneke [c], V. Nelen [d], N. van Larebeke [e], R. Vlietinck [f], G. Schoeters [a]

[a] *Flemish Institute of Technological Research (VITO), Center of Expertise in Environmental Toxicology and Environmental Measurements, 2400 Mol, Belgium*
[b] *Toxicological Center, University of Antwerp, Universiteitsplein 1, 2610 Antwerpen (Wilrijk), Belgium*
[c] *Medical Institute of Environmental Hygiene, Heinrich-Heine University, 40225 Düsseldorf, Germany*
[d] *Department of Epidemiology, Provincial Institute of Hygiene, 2000 Antwerp, Belgium*
[e] *Department of Radiotherapy, Nuclear Medicine and Experimental Oncology, University of Ghent, 9000 Ghent, Belgium*
[f] *Centre of Human Genetics, University of Leuven, 3000 Leuven, Belgium*

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Abstract

In 1999, a campaign of the Flemish Ministry of Health, Belgium was set up to assess pollutant concentrations and related health effect biomarkers in humans living in two regions of Flanders. The study was called the 'Flemish Environment and Health Study' (FLEHS). One of the goals was to measure present concentrations of persistent organochlorine pollutants in a Flemish population and to compare values obtained from pooled and individual serum samples. Concentrations of selected organochlorine pesticides, polychlorinated biphenyls (PCB) and polychlorinated dibenzo-*p*-dioxins (PCDD) and furans (PCDF) were measured by gas chromatography–mass spectrometry. TEQ values were also assessed by Chemical-Activated LUciferase gene eXpression (CALUX) bioassay. The study population consisted of 200 women between 50 and 65 years living in two areas of Flanders, Belgium. Because of the large volumes serum needed for all measurements, the concentrations of organochlorines were measured in 47 pooled serum samples originating from these women. The concentrations of the indicator PCBs (359.8 ng/g fat) and organochlorine pesticides (hexachlorobenzene, *p,p′*-dichlorodiphenyldichloroethylene, *p,p′*-dichlorodiphenyl-trichloroethane, lindane and pentachlorophenol), were comparable to those found in other European countries. The concentrations of PCDD/PCDFs showed another picture. With a median value of 48 pg WHO-TEQ/g fat, the women had 2-fold higher levels than a comparable age group from Germany examined in 1996. The mean total WHO-TEQ including PCDD/F, non-ortho and mono-ortho PCBs was 72.7 pg WHO-TEQ/g fat, whereas the CALUX-TEQ mean value was only 35.0 pg TEQ/g fat. In order to assess the pooling procedure, indicator PCBs and CALUX-TEQs were measured in all 200 individuals that were integrated in the pools. The measured values were comparable to the pool results: 390.0 ng/g fat and 41.6 pg TEQ/g fat respectively. It was concluded that pooling of serum samples offers the possibility to measure exposure in the whole study population on a more cost-effective way. However, because of statistical power loss and no possibility of confounder adjustment, pooling is not the most effective way to study regional differences.

* Corresponding author. Fax: +32-3-820-2722.
E-mail address: covaci@uia.ua.ac.be (A. Covaci).

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1. Introduction

Persistent organochlorine pollutants (POPs) represent a class of widespread environmental contaminants including polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and polychlorinated biphenyls (PCBs). Due to potential health effects (dermal toxicity, immunotoxicity, reproductive effects and teratogenicity, endocrine disruption and carcinogenicity), their monitoring in humans is of high-general concern (WHO, 1998). Enough information on POPs presence in human tissues from industrialised countries is available to suggest that the concentration of these compounds have decreased during the last 10 years (Van Cleuvenbergen et al., 1994; Ewers et al., 1996; EU Dioxin Exposure Data, 1999).

Few analyses of POPs in the Belgian population have been done until now. It was shown that PCDD/PCDF concentrations in pooled mother milk from Brabant-Walloon (rural), Brussels (urban) and Liège (industrial) collected in 1988 were among the highest in the world (Tarkowski and Yrjänheikki, 1989). The mean value of these three pooled human milk samples was 37.6 pg I-(toxicity equivalents, TEQ)/g fat. In 1993, three pooled samples from the same regions were measured. The mean concentration was now 24.8 pg I-TEQ/g fat, which means an average decrease of 34% from 1988 to 1993 (EU Dioxin Exposure Data, 1999). In 1992, nine randomly chosen individual human milk samples from five Flemish provinces were analysed. The average TEQ value was 34.4 pg I-TEQ/g fat with an individual range between 27.3 and 43.2 pg I-TEQ/g fat (Van Cleuvenbergen et al., 1994). In a more complete study, four PCB congeners (IUPAC nos. 118, 138, 153 and 180) were analysed in serum samples collected between 1996 and 1998 from 106 infertile women visiting three university hospitals from Flanders (Pauwels et al., 2000a,b). The average sum of the four PCB congeners was 263.5 ng/g fat. Analysis of dioxin-like compounds in the same serum samples using the Chemical-Activated LUciferase gene eXpression (CALUX) in vitro bioassay gave the mean result of 46.8 pg TEQ/g fat (Pauwels et al., 2000a). In Wallonia (Belgium), a study was set up in 1999 to assess PCDD/PCDF and heavy metal loads in the general population. A mean PCDD/PCDF concentration of 36.7 pg I-TEQ/g fat was found in a group of 54 men and women (mean age of 50 years) living near to a municipal waste incinerator. This was 34% higher than the mean concentration (27.2 pg I-TEQ/g fat) measured in 32 persons of same age range living in a rural area (Bernard, personal communication).

In parallel, a detailed study was set up by the Flemish Government. A goal of this research was the evaluation of concentrations of heavy metals, polyaromatic hydrocarbons (PAH), volatile organic compounds (VOC) and POPs in populations from two regions in Flanders, Belgium. Beside biomonitoring of exposure, some effects on DNA, immunological and renal system, bone metabolism and fertility were studied by the use of effect biomarkers (Staessen et al., 2001; Van Loon et al., 2002).

This article describes the analysis of selected POPs, including PCDD/PCDFs, PCBs and organochlorine pesticides, in the serum of 200 healthy women (50–65 years). Primary objectives of these analyses were: (i) determination of mean concentrations and regional differences of these pollutants based on pooled and individual results, (ii) calculation of correlations between concentrations of polychlorinated hydrocarbons measured in human serum and evaluation of potential markers, (iii) comparison of CALUX-TEQ with chemically measured TEQs in human biomonitoring studies. This is the first study with such amplitude done in Belgium. The first objective of this study is discussed further, while the other topics will be presented elsewhere.

2. Materials and methods

2.1. Study area

The rural area of Peer is situated 15–25 km from the nearest non-ferrous and chemical plants and lies away from motorways. The urban area (two suburbs of Antwerp city) is located 11–13 km SE from the chemical and petrochemical industry established in the harbour, but close to a non-ferrous smelter, two municipalwaste incinerators, a crematory, printing works, several small or medium-sized enterprises and a major motorway. The two waste incinerators have been in operation from 1971 to 1980, respectively. In 1997, they had annual turnovers of 23 000 and 110 000 tons. They were shut down in November 1997, because PCDD/PCDFs emissions had exceeded the limit value of 0.1 ng I-TEQ/N m^3 (Schoeters, 1998). In the past, emissions of PCDD/PCDFs (one yearly measurement) were between 2 and 7 ng I-TEQ/N m^3. In 1998, concentrations of PCDD/PCDFs in soil samples (0–5 cm depth) close to the incinerators ranged between 3.5 and 35.9 ng I-TEQ/kg dry weight (De Fré et al., 1999).

2.2. Study group

The study group consisted of 200 healthy women from Antwerp ($n = 100$) and Peer ($n = 100$) recruited between June and September 1999. This age group was selected because, due to bioaccumulation, elderly people have a higher POP body burden that theoretically would facilitate comparison of all different analyses. The initial selection comprised of 2898 randomly selected women between 50 and 65 years old, which were contacted by letter. About half of the 40.1% and 30.8 % responders in Antwerp and Peer respectively, were further selected ($N = 685$) because of compliance to the following four criteria: non- or ex-smoker, minimal residence time of 10 years in the study area, working in the town of residence or at home and exclusion of jobs with specific risks of exposure. From those selected women, 255 were contacted by telephone, and 200 individuals decided to participate in the study. Each participant filled in an informed consent and the study was approved by the Ethical Committee of the University of Leuven.

Dietary information was obtained by a semi-quantitative food frequency questionnaire on meat, fish, eggs, milk and cheese. These data reflected only the consumption habits of last year, but they were considered to be indicative for the food consumption during the past years. In order to estimate the dietary intake of PCBs and dioxins, fat scores and dairy consumption frequencies were calculated. Fat scores were calculated taken into account the average amount per consumption event and the average fat content for each food group, according to the Dutch food composition table (Van Erp-Baart, 1993). Consumption frequencies of milk, cheese and eggs were added and expressed as times of dairy intake per day. In case of outliers in the food categories, the upper and lower categories of intake were collapsed until there were at least five observations in the category representing the highest respectively the lowest frequencies. Because the concentrations of investigated compounds in locally produced food may vary by region, information on the consumption of locally produced food was requested. The body mass index (BMI), expressed as weight (kg) divided by the square of the height (m) was calculated for each individual.

2.3. Sample collection and pooling procedure

Approximately 40 ml of blood was collected from each individual. Blood samples were collected in polyethylene recipients. Immediately after sampling, serum was separated and divided into one part for individual analysis of indicator PCBs congeners (3 ml) and CALUX-TEQ (2.5 ml) and another part for pooling. Pooling was done by ranking the women in the order of decreasing daily intake of meat and fish, decreasing daily intake of eggs and milk, increasing total number of weeks of lactation and increasing BMI. This was done practically by sorting the data first on BMI (for this age group considered to be the least important criteria), followed by a second sorting round on the weeks of lactation (the second least important criteria), and so on. The available serum of 3–5 subsequently listed individuals was pooled to approximately 50 ml. Each of the 47 pooled samples was divided in three aliquots for the analysis of PCDD/PCDFs (25 ml), PCBs/organochlorine pesticides (13 ml) and CALUX-TEQ (4 ml). All samples were stored in glass vials precleaned with hexane and acetone, and kept at −20 °C until analysis.

2.4. Target analytes

The analysed compounds are listed in Table 1. In 200 individual serum samples, the indicator PCBs and CALUX-TEQ values were determined. In 47 pooled serum samples, the following PCB congeners were measured: mono-ortho PCBs (PCB 105, 118, 156, 157, 167), indicator PCBs (28, 52, 101, 138, 153, 180) and PCB 44, 66, 74, 99, 110, 128, 149, 170, 183, 187, 194, 199. The pooled samples were also analysed for the non-ortho PCBs (77, 81, 126, 169) and the 17 PCDD/PCDF toxic congeners. The analysis of mono-ortho, non-ortho PCBs and PCDD/PCDF congeners allowed the calculation of the TEQ for each sample using the TEF scheme (Table 1) of the WHO Van den Berg et al., 1998. Hexachlorobenzene (HCB), p,p'-dichlorodiphenyldichloroethylene (p,p'-DDE), p,p'-dichlorodiphenyl-trichloroethane (p,p'-DDT), lindane (γ-HCH) and pentachlorophenol (PCP) were also measured in the pooled samples as major organochlorine pesticides found in human serum. For all compounds, measurements under the detection limit were set at half of this detection limit ('medium bound' method). The chemical analysis methods have been previously described in detail and are briefly summarised below. Serum concentrations of triglycerides (TG), cholesterol (CH) and phospholipids (PL) were determined enzymatically as presented in Pauwels et al. (2000b). The lipid adjustment was done by dividing fresh weight concentrations (ng/ml) to the total lipid content (calculated as the sum of TG, CH and PL). All solvents were of pesticide grade purity and were used without any further treatment.

2.5. Analysis

2.5.1. Indicator PCBs

The analysis of indicator PCBs (Table 1) was done by a previously described method (Fastabend, 2000) using high-resolution gas chromatograph (HRGC) with electron capture detection (ECD) equipped with two

Table 1
Overview of the persistent polychlorinated compounds analysed in 47 pooled serum samples originating from 200 women (50–65 years)

Compound[a]	TEF-value[b]	Serum sample (*N*)	Analysis[c]
Indicator PCBs		Pool (47)	GC-LRMS
PCB 28, 52, 101, 138, 153, 180	–	Individual (200)	GC-ECD
Mono-ortho PCBs		Pool (47)	GC-LRMS
PCB 105	0.0001		
PCB 118	0.0001		
PCB 156	0.0005		
PCB 157	0.0005		
PCB 167	0.00001		
Non-ortho PCBs		Pool (47)	GC–HRMS
PCB 77	0.0001		
PCB 81	0.0001		
PCB 126	0.1		
PCB 169	0.01		
Other PCBs		Pool (47)	GC-LRMS
PCB 44, 66, 74, 99, 110, 128, 149, 170, 183, 187, 194, 199	–		
PCDF/PCDDs		Pool (47)	GC–HRMS
2,3,7,8-T_4CDF	0.1		
1,2,3,7,8-P_5CDF	0.05		
2,3,4,7,8-P_5CDF	0.5		
1,2,3,4,7,8-H_6CDF	0.1		
1,2,3,6,7,8-H_6CDF	0.1		
2,3,4,6,7,8-H_6CDF	0.1		
1,2,3,7,8,9-H_6CDF	0.1		
1,2,3,4,6,7,8-H_7CDF	0.01		
1,2,3,4,7,8,9-H_7CDF	0.01		
O_8CDF	0.0001		
2,3,7,8-T_4CDD	1		
1,2,3,7,8,P_5CDD	1		
1,2,3,4,7,8-H_6CDD	0.1		
1,2,3,6,7,8-H_6CDD	0.1		
1,2,3,7,8,9-H_6CDD	0.1		
1,2,3,4,6,7,8-H_7CDD	0.01		
O_8CDD	0.0001		
Calux bioassay		Pool (47)	CALUX
TEQ-parameter	–	Individual (197)	
Pesticides		Pool (47)	GC-LRMS
HCB			
PCP			
p,*p*′-DDE			
p,*p*′-DDT			
γ-HCH			

CALUX and marker PCB analyses were performed also on the individual samples.
[a] IUPAC numbering.
[b] Toxic equivalency factors from Van den Berg et al. (1998).
[c] LRMS—low resolution mass spectrometer, HRMS—high-resolution mass spectrometer, ECD—electron capture detection.

capillary columns of different polarity. Briefly, the samples (2 ml) were mixed with formic acid for homogenisation, followed by a two step liquid extraction of PCBs with *n*-heptane and purification of extracts by a silica gel column. The PCBs were identified by their retention times and quantification was carried out using mirex as internal standard. The detection limit for each congener was 0.015 ng/ml and recoveries were between 75% and 85%. For internal quality control, a blind and a control sample were included in each series of mea-

surements, while the external quality control was ensured by participation to interlaboratory tests.

2.5.2. PCB and pesticide analysis

The analysis of organochlorine pesticides and PCB congeners (Table 1) was done on 47 pooled samples. Details of the procedure were previously described (Covaci and Schepens, 2001). Briefly, 10 ml of serum were spiked with internal standards PCB 46 and 143 and mixed with formic acid. The mixture was subjected to extraction using solid phase disk extraction cartridges, followed by clean-up on acidified silicagel. Analyses were performed using a GC-LRMS equipped with a 50 mm × 0.22 mm HT-8 capillary column (SGE, Zulte, Belgium). A selected ion monitoring table was constructed for quantification. Three ions (two from molecular ion cluster M^+ and $[M+2]^+$ and the $[M-70]^+$ ion) were monitored for each level of chlorination for PCBs or for each pesticide. Detection limits were 0.03 ng/ml (HCB), 0.02 ng/ml (γ-HCH), 0.04 ng/ml (p,p'-DDE), 0.015 ng/ml (p,p'-DDT) and ranged between 0.01 and 0.02 ng/ml for individual PCBs. When expressed based on lipid weight, limits of detection for selected compounds were between 1 and 2 ng/g fat. Recoveries for selected compounds ranged from 62% to 74%. Quality control was ensured by regular analysis of an in-house control serum, procedural blanks and participation to interlaboratory tests.

For PCP analysis, ^{13}C-PCP (internal standard) was added to 0.5 ml serum mixed with formic acid and extracted with hexane. After concentration, the extract was derivatised with diazomethane (to form Me-PCP) and purified on acid silica micro-column. After concentration of the eluate, 1 μl was injected in a GC–MS in splitless mode. Recovery of PCP was 72 ± 6%. The detection limit for PCP was 0.07 ng/ml serum or 10 ng/g fat.

2.5.3. PCDD, PCDF and non-ortho PCB analysis

Approximately 20 g human serum was mixed with isopropanol and spiked with internal standard solutions, containing the ^{13}C-labelled compounds (PCDD/PCDFs and non-ortho PCBs). After homogenisation, 45 g of silica and 45 g of anhydrous sodium sulphate were added, grounded to a fine free-flowing powder and extracted for 5 h in a Soxhlet apparatus with *n*-hexane:acetone (2:1, v/v). A first clean-up of the concentrated extract was performed on a multilayer chromatographic column filled with, from bottom to top, 2 g of silica/NaOH 33% 1 N, 10 g of silica/H_2SO_4 44% and 10 g of anhydrous sodium sulphate. After elution with hexane and concentration, the extract was purified by HPLC on a Hypercarb column and on alumina column. The cleaned extract was concentrated and transferred to a vial. The analysis was performed on a Micromass Autospec Ultima GC–HRMS, equipped with a splitless injector and a 60 m × 0.25 mm DB5-ms capillary column (J&W Scientific, Folsom, CA, USA). The HRMS was operated in the electron impact ionisation mode at 34 eV with a resolution of 6000 and the acquisition was based on selected ion recording, using six groups which contained the two most intense ions of the molecular ion cluster of each compound. Identification of the analytes relied on the mass peak, the relative retention time and the calculated vs. theoretic isotopic ratio for the masses monitored per compound.

For quality control purposes, a procedure blank starting from 20 ml of ultrapure water was included with each series of five serum samples. Blanks averaged at 0.08 and 0.14 pg TEQ/g serum for PCDD/PCDFs and non-ortho PCBs, respectively. Because of the small amounts of serum available, the blank contribution was non-negligible and sample results had to be corrected for it. In particular for PCB 126, this might have led to a larger uncertainty of the data. The recovery of the ^{13}C-labelled internal standards was in the range of 70–130%. A milk powder reference material (CRM 534) was analysed with the samples in 3-fold. The recovery on TEQ basis amounted to 95–102%. The laboratory has frequently and successfully participated to interlaboratory studies, e.g. Dioxin in Food (Folkehelsa, NO) and SETOC (WEPAL, NL), and to the certification of BCR reference materials for PCDD/PCDFs and PCBs (CRM 490, 607, 615 and 677). It has been accredited according to EN 45001 for the analysis of PCDD/PCDFs in emissions, milk and dairy products.

2.5.4. CALUX bioassay

Polychlorinated chemicals in serum were estimated with a CALUX assay variant based on a previously described procedure (Murk et al., 1998). In this assay, dioxin-like compounds are assessed via in vitro activation of the aryl hydrocarbon receptor (AhR) of cultured H4IIE cells. The method involves *n*-hexane extraction of blood serum (4 ml from the pooled serum and 2 ml from the individual samples) and removal of matrix components by passage through a 33%H_2SO_4 silica column. The extract was partly evaporated and quantitatively transferred to a conical vial for further evaporation. It was reconstituted in dimethyl sulphoxide (DMSO, Acros Organics) for CALUX measurement using rat hepatoma H4IIE cell line which is transfected with an AhR-controlled luciferase reporter gene construct. Cells were grown in 96-well plates in 100 μ1 minimal essential medium (α-MEM, Gibco) with 10% fetal calf serum (FCS, Gibco) at a temperature of 37 °C and 5% CO_2. When the cell layer reached 70–80% confluency, the samples and TCDD standards were dosed in quadruplicates to the cells for 24 h. After removal of the medium, cells were washed with 100 μl phosphate-buffered

saline without Ca/Mg (PBS-Ca/Mg, Life Technologies) and 30 μl of cell lysis reagent (Promega) was added. The well plates were then shaked for at least 45 min and stored at −80 °C for at least 1 h. For determination of luciferase activity, the cells were thawed on ice, and 100 μl luciferin assay mix (Promega) was added at room temperature. The light production was measured by a Victor 2 Luminometer (EG&G Wallac). The CALUX-based TEQs were calculated by comparing the luciferase activity induced by the sample against a dose-response curve generated from 2,3,7,8-T_4CDD concentration standards analysed simultaneously. The limit of detection varied with cell growth and volume of the blood samples. This detection limit was calculated as the signal measured from the DMSO solvent control on each well plate plus three times its standard deviation. For 4 ml serum with 700 mg fat/dl the limit of detection was 5.2 ± 3.5 pg TEQ/g fat. Measurements below were set at half of the detection limit. A fetal calf serum sample was run for each series of study samples as internal standard. The interexperiment variation was less than 30% and accepted as normal for this low-loaded sample.

2.6. Statistical analysis

Database management and statistical analysis were performed with Statistica version'99 (Statsoft Inc.). Analytical data that were not normally distributed were log transformed. Means and proportions were compared across the two areas by *t*-test (or alternative Mann–Whitney *U*-test) and χ^2 test, respectively. The statistical methods also included analysis of covariance to adjust for confounders in the interregional comparison. Personal attributes such as age, fasting status, BMI, number of children, lactation history, and food consumption behaviour (intake dairy, fat, local food) were considered in the multivariate models to assess regional differences in the individual measurements. This was not possible for regional comparison of pooled data. The statistical power of the analysis test was calculated being the certainty of not finding false negative results when comparing mean concentrations of two regions. It was calculated for the respective number of samples analysed, based on the formula for comparison of two groups of normally distributed values.

3. Results

3.1. Study group

The study group had an average age of 58.5 years. There were no differences in age or anthropometric characteristics (BMI = 26) between the women living in the rural area of Peer and the urban area of Antwerp (Table 2). However, the women from Peer were in average working for about 30 years, whereas in Antwerp this was only 10 years. The consumption of dairy products was slightly higher in Peer, while the local product consumption was significantly higher: 72% of women in Peer versus 28% in Antwerp. The number of children and months of breastfeeding were significantly higher in Peer (Table 2).

3.2. Pooled samples versus individual serum samples

Serum concentrations of indicator PCBs and CALUX-TEQ values were measured in each of the 200 women (Table 3). As for the pooled samples, concentrations of the indicator PCBs were significantly higher in the urban area (423.6 versus 362.8 ng/g fat, $p = 0.002$), when adjusted for age, animal fat and dairy consumption, and fasting status. Assessing regional differences without adjustment for confounding factors resulted in a lower significance of the difference (424.8 versus 370.3.1 ng/g fat, $p = 0.02$). The individual CALUX-TEQ values were different from the results from the pooled samples. Whereas there was no regional difference observed for the pooled samples, the individual CALUX-TEQ values were significantly higher in Peer (44.2 pg TEQ/g fat) compared to Antwerp (32.1 pg TEQ/g fat) ($p = 0.03$), when adjusted for number of weeks lactation. This difference was more significant for non-adjusted measurements ($p = 0.002$).

3.3. Concentrations of POPs and regional differences for pooled serum samples

Concentrations of some organochlorine pesticides (PCP, p,p'-DDE and γ-HCH) were higher (but not significantly) in Peer, while the concentration of p,p'-DDT was significantly higher in that region. HCB concentration was significantly higher in Antwerp (130.1 versus 92.2 ng/g fat, $p = 0.001$).

Three PCB congeners (IUPAC nos. 138, 153 and 180) were present in all pooled samples and contributed with approximately 65% to the total PCB concentration (sum of 27 congeners). Their concentration was significantly higher in the urban region (391.9 ng/g fat versus 334.4 ng/g fat) (Table 3). Concentrations of other congeners such as PCB 118 and 156 were also significantly higher in the urban region (36.7 versus 23.5 ng/g fat for PCB 118 and 17.9 versus 14.1 ng/g fat for PCB 156, respectively). Concentrations of PCB 170, 180, 187, 194 and 199 were not significantly different between the two regions. The total PCB concentration was significantly higher in Antwerp (572.5 versus 493.9 ng/g fat, $p < 0.005$).

The mean TEQ concentrations were 24.2 and 22.1 pg/g TEQ/g fat for PCDDs and PCDFs, respectively.

Table 2
Description of studied population

Characteristics	Peer $n = 100$		Antwerp $n = 100$		p^*
	Mean (SD)	Median	Mean (SD)	Median	
Clinical measurements					
Length (cm)	158 (6)	157	159 (6)	159	NS
Weight (kg)	69 (12)	67	68 (13)	66	NS
BMI (kg/m^2)	27.5 (4.4)	26.4	26.6 (5.1)	26.2	NS
Blood fat (mg/dl)	712 (139)	692	714 (165)	705	NS
Data from the questionnaires					
Age (years)	58.4 (4.1)	59.0	57.8 (4.1)	58.0	NS
Number of years living in the region	42 (14)	41	38 (14)	35	NS
Number of years working	27 (13)	32	10 (10)	8	<0.001
Ex-smokers (%)	7.1		9.7		NS
Number of years stopped smoking	34.0 (9.3)	30.0	26.9 (6.4)	28.0	NS
Passive smoking (h/day)	0.8 (2.2)	0.0	2.5 (4.6)	0.0	0.04
Dairy consumption (frequency/day)[a]	0.49 (0.25)	0.45	0.48 (0.26)	0.43	NS
Consumption of animal fat[b] (score, g/day)	0.55 (0.20)	0.53	0.56 (0.23)	0.53	NS
Consumption of local food (%)	72		28		<0.001
Total number of breastfeeding weeks	22 (34)	10	8 (22)	0	<0.001
Number of children	2.9 (1.6)	3.0	2.0 (1.4)	2.0	<0.001
Age at birth of first child (years)	24.6 (3.8)	23.9	23.8 (3.6)	23.1	NS

NS—not significant for $p > 0.05$. *Significant difference ($p < 0.05$) between the two regions determined with the Mann–Whitney U-test or χ^2-test for 2×2 tables.

[a] Average for milk, cheese and eggs.

[b] Fat from fish, shrimps, mussels, meat, cheese, milk, eggs, based on the average daily consumption and average fat content of each nutrition group.

When non- and mono-ortho PCBs were added, the mean total WHO-TEQ value increased to 72.7 pg/g TEQ/g fat. There was no statistical difference in the PCDD or PCDF concentrations in the two regions, except for 1,2,3,4,6,7,8-H_7CDD, which was slightly significantly higher in Antwerp ($p = 0.05$). Most of PCDD concentrations were slightly higher in Antwerp, while most of PCDF concentrations were higher in Peer. Non- and mono-ortho PCB-TEQ values were significantly higher in the urban area (Table 3), while the PCDD/PCDF-TEQ and the total WHO-TEQ (sum of PCDDs, PCDFs, non-ortho and mono-ortho PCBs) were not statistically different for both regions. PCDDs and PCDFs contributed almost equally to the PCDD/PCDF-TEQ (average of 53% and 47%, respectively). The mean PCDD/PCDF contribution to the total WHO-TEQ value was 67%. The non- and mono-ortho PCBs contributed to the total WHO-TEQ with 16% and 17%, respectively. The principal contributors to the total WHO-TEQ value were 2,3,4,7,8-P_5CDF (PCDFs), 1,2,3,7,8-P_5CDD (PCDDs), PCB 126 (non-ortho PCBs) and PCB 156 and 118 (mono-ortho PCBs). Dioxin-like toxicity was also assessed by the CALUX bioassay (mean value of 35.0 pg TEQ/g fat). No significant regional difference was observed in dioxin-like toxicity of the pooled serum samples (35.8 pg-TEQ/g fat in Peer versus 34.4 pg-TEQ/g fat in Antwerp, $p = 0.61$).

4. Discussion

4.1. Analysis of pooled versus individual serum samples

Pooling of serum samples was done because of large volumes of serum needed for all analyses and to reduce the number of samples to be analysed, while keeping the resulting analytical information at acceptable levels. To the best of our knowledge, no other study has addressed the measurement of indicator PCBs and CALUX-TEQs on serum pools as well as on the individuals constituting the pools. The mean concentrations of indicator PCBs and CALUX-TEQs from 47 pools and 200 individuals were comparable. The geometric mean of indicator PCBs and CALUX-TEQs in pools and individuals was 360 and 390 ng/g fat, and 35 and 41.6 pg TEQ/g fat respectively. As it can be seen in Table 3, the concentration of PCB 138 was somewhat higher for the analyses done on individual samples. This was due to the use of a Ultra-2 GC column for the individual samples (on which PCB 138 co-elutes with PCB 163 and 164), whereas for the pooled samples, the separation of PCB 138 from PCB 163 was done by a HT-8 column.

Depending on the amount of serum obtained, a pool consisted of 3–17 ml serum from four persons in average. The pooling procedure lead to the smoothing of extreme values and the loss of significance for region comparison.

Table 3
Median concentrations (95th percentiles) of POPs measured in pooled and individual serum samples of 200 women (50–65 years) living in two regions of Flanders, Belgium

Compound[a]	Pool ($N_{pools} = 47$)				Individuals ($N = 200$)			
	Peer + Antwerp	Peer ($N = 22$)	Antwerp ($N = 25$)	p^*	Peer + Antwerp	Peer ($N = 100$)	Antwerp ($N = 100$)	p^*
Indicator PCBs (ng/g fat)								
PCB 28	nd	nd	nd	–	nd	nd	nd	
PCB 52	nd	nd	nd	–	nd	nd	nd	
PCB 101	nd	nd	nd	–	nd	nd	nd	
PCB 138	91.8 (76.7–107.3)	83.4 (73.0–100.4)	104.7 (90.1–118.1)	**0.001**	116.9 (87.2–151)	108.5 (81.7–130.1)	127.6 (96–173.2)	**<0.001**[b]
PCB 153	167.6 (145.5–201.8)	150.2 (139.0–175.0)	180.5 (165.6–211.2)	**0.008**	158.1 (123.0–210)	146.8 (117.0–179.4)	175.2 (132.2–230.4)	**<0.001**[b]
PCB 180	104.1 (89.2–120.7)	100.2 (89.2–110.3)	109.3 (98.7–125.6)	0.42	115.4 (94.2–141)	110.3 (92.2–137.1)	120.1 (97.0–151.4)	0.15[b]
Sum indicator PCBs	359.8 (308.4–430.7)	334.4 (308.0–371.7)	391.9 (358.4–451.7)	**0.020**	390.0 (309.6–500)	370.3 (295.0–437.1)	424.8 (330.0–551.5)	**0.002**[b]
Mono-ortho PCBs (ng/g fat)								
PCB 105	7.1 (5.8–9.4)	5.9 (5.3–7.1)	7.4 (6.6–9.8)	**<0.001**		–	–	–
PCB 118	29.2 (23.1–38.0)	23.5 (20.9–29.2)	36.7 (29.2–40.7)	**<0.001**		–	–	–
PCB 156	15.8 (13.9–18.1)	14.1 (12.8–16.1)	17.9 (14.7–19.8)	**0.007**		–	–	–
PCB 157	2.6 (1.5–3.1)	2.6 (1.5–3.1)	2.6 (1.4–3.1)	0.99		–	–	–
PCB 167	0.8 (0.7–7.9)	0.8 (0.7–2.9)	5.9 (0.8–8.7)	**0.042**		–	–	–
Mono-ortho PCB TEQ (pg/g fat)	11.8 (11.1–15.2)	11.3 (10.4–12.1)	14.5 (11.7–16.8)	**0.001**		–	–	–
Non-ortho PCBs (pg/g fat)								
PCB 77	nd	nd	nd	–		–	–	–
PCB 81	nd	nd	nd	–		–	–	–
PCB 126	101.9 (82.2–151.5)	92.5 (78.8–116.5)	144.6 (96.9–174.5)	**0.005**		–	–	–
PCB 169	112.4 (104.3–126.2)	114.4 (105.5–125.6)	111.6 (98.8–131.0)	0.90		–	–	–
Non-ortho PCB TEQ	11.9 (9.7–17.0)	10.6 (9.2–13.0)	16.1 (11.1–19.0)	**0.006**		–	–	–
Other PCBs (ng/g fat)								
PCB 44	nd	nd	nd	–		–	–	–
PCB 66	nd	nd	nd	–		–	–	–
PCB 74	13.8 (11.4–17.9)	11.7 (9.9–13.8)	17.6 (13.3–21.3)	**<0.001**		–	–	–
PCB 99	14.5 (10.2–18.1)	11.9 (9.5–15.5)	17.9 (13.7–20.5)	**0.001**		–	–	–
PCB 110	nd	nd	nd	–		–	–	–
PCB 128	nd	nd	nd	–		–	–	–
PCB 149	nd	nd	nd	–		–	–	–
PCB 170	40.1 (37.6–46.8)	38.8 (37.6–41.9)	43.8 (38.3–49.8)	0.11		–	–	–
PCB 183	8.8 (7.1–10.2)	7.7 (6.2–9.3)	9.2 (8.1–11.0)	**0.008**		–	–	–
PCB 187	18.9 (14.2–24.8)	18.1 (14.7–19.0)	22.1 (13.8–27.7)	0.13		–	–	–
PCB 194	14.6 (13.3–17.5)	14.1 (13.4–15.3)	15.7 (13.3–17.8)	0.94		–	–	–
PCB 199	16.0 (13.9–19.1)	15.6 (13.9–17.5)	16.3 (14.4–19.6)	0.50		–	–	–
Sum PCBs (ng/g fat)	530.2 (454.5–677.7)	493.9 (452.7–558.7)	572.5 (515.9–723.7)	**0.005**		–	–	–

PCDFs + PCDDs (pg/g fat)								
2,3,7,8-T_4CDF	nd	nd	nd	–		–	–	–
1,2,3,7,8-P_5CDF	3.1 (2.4–5.0)	3.4 (2.6–4.4)	2.9 (2.2–5.9)	0.44		–	–	–
2,3,4,7,8-P_5CDF	30.8 (24.6–38.1)	31.5 (24.6–38.5)	30.8 (26.0–37.6)	0.86		–	–	–
1,2,3,4,7,8-H_6CDF	12.2 (10.0–15.5)	13.8 (10.8–17.1)	11.8 (9.0–13.1)	0.18		–	–	–
1,2,3,6,7,8-H_6CDF	10.7 (9.0–15.1)	11.5 (8.4–15.1)	10.3 (9.2–14.9)	0.63		–	–	–
2,3,4,6,7,8-H_6CDF	5.7 (4.5–8.8)	5.8 (4.7–7.5)	5.7 (4.0–8.8)	0.40		–	–	–
1,2,3,7,8,9-H_6CDF	2.3 (1.6–4.6)	2.6 (1.7–5.8)	2.3 (1.6–3.7)	0.44		–	–	–
1,2,3,4,6,7,8-H_7CDF	11.6 (9.9–15.2)	11.3 (9.5–12.2)	12.2 (11.4–16.6)	0.17		–	–	–
1,2,3,4,7,8,9-H_7CDF	5.1 (3.3–8.1)	5.2 (4.0–10.1)	4.6 (3.3–6.7)	0.31		–	–	–
O_8CDF	14.9 (11.4–28.6)	19.4 (12.4–60.3)	13.1 (11.2–16.9)	0.07		–	–	–
2,3,7,8-T_4CDD	4.9 (3.4–6.0)	4.7 (3.1–6.1)	5.3 (4.3–5.9)	0.39		–	–	–
1,2,3,7,8-P_5CDD	13.1 (10.8–16.2)	12.8 (10.7–14.6)	13.2 (11.9–16.3)	0.42		–	–	–
1,2,3,4,7,8-H_6CDD	10.8 (9.3–12.1)	10.7 (9.3–12.1)	11.4 (9.6–12.0)	0.74		–	-	–
1,2,3,6,7,8-H_6CDD	42.7 (38.6–50.8)	41.9 (39.4–50.8)	42.9 (38.1–47.0)	0.83		–	–	–
1,2,3,7,8,9-H_6CDD	8.5 (6.1–10.2)	7.6 (5.8–9.7)	8.7 (6.6–10.2)	0.28		–	–	–
1,2,3,4,6,7,8-H_7CDD	79.2 (57.0–95.9)	77.4 (53.8–87.7)	79.3 (64.4–98.6)	**0.05**		–	–	–
O_8CDD	743.8 (656.9–945.8)	752.1 (677.4–945.8)	736.0 (619.5–933.7)	0.96		–	-	–
PCDFs TEQ	22.1 (18.7–27.7)	22.2 (18.6–27.2)	22.1 (19.7–27.9)	0.95		–	–	–
PCDDs TEQ	24.2 (21.6–29.9)	24.2 (21.2–30.0)	24.2 (22.6–29.7)	0.33		–	–	–
PCDFs + PCDDs TEQ	48.0 (41.3–57.2)	48.9 (41.0–54.5)	44.6 (42.1–58.3)	0.68		–	–	–
PCDDs/PCDFs TEQ	1.2 (1.0–1.3)	1.2 (1.0–1.3)	1.2 (1.0–1.3)	0.50		–	–	–
Total WHO-TEQ (pg TEQ/g fat)	72.7 (66.0–85.8)	72.6 (61.7–77.2)	75.1 (68.2–93.2)	0.06		–	–	–
Calux bioassay (pg TEQ/g fat)	35.0 (24.4–48.1)	35.8 (26.6–46.0)	34.4 (23.4–49.1)	0.61	41.6 (18.9–63.2)	44.2 (30.6–64.9)	32.1 (11.6–60.0)	**0.03**[b]
Pesticides (ng/g fat)								
HCB	109.9 (89.6–132.0)	92.2 (84.5–113.2)	130.1 (107.4–142.6)	**0.001**		–	–	–
PCP	713.7 (395.4–960.3)	803.5 (500.0–976.9)	483.4 (390.6–837.0)	0.08		–	–	–
p,p′-DDT	2.6 (1.1–4.4)	3.7 (2.7–5.0)	1.1 (1.1–2.6)	**0.003**		–	–	–
p,p′-DDE	871.3 (725–1202.4)	944.9 (834–1283.2)	832.6 (691–1130.0)	0.46		–	–	–
γ-HCH	5.7 (1.6–8.1)	6.0 (4.3–8.4)	4.9 (1.5–6.5)	0.06		–	–	–

(–) Not measured, nd: not detectable (below detection limit) in all or nearly all samples. *p value for regional comparison of the data expressed per g serum fat.

[a] IUPAC numbers.

[b] Regional comparison of the individual data was done taking into account the covariates: number of lactation weeks (for CALUX-TEQ) or age, fasting status, and fat and dairy consumption frequency (for indicator PCBs).

In our study, regional differences were not observed when comparing the CALUX-TEQs of the pools ($p = 0.61$). However, when comparing CALUX-TEQs of the individual samples, a significant difference was found between the two regions (Peer > Antwerp, $p = 0.03$) (Table 3). This could not be explained through confounder adjustment, which was possible only for the individual samples. The nutritional and life style parameters obtained where not powerful enough to be of much influence. The statistical power for comparison of 25 pools or 100 individual samples in both regions increased from 66% to 78% for the indicator PCB assessment, and from 8% to 97% for the CALUX measurement. It is clear that there was a loss of power when pooling 100 individuals into 25 samples per region. Therefore, pooling should be avoided if the purpose of the study is to compare pollutant concentrations between different areas. Besides loss of information (less statistical power, thus more samples needed), there is no possibility to adjust for possibly essential confounders like age, antropometric characteristics and food consumption. On the other hand, if the mean concentration of POPs in human serum for all people in both regions is of interest, the pooling procedure offers a good and cheaper alternative.

4.2. Serum POP values of Flemish women: comparison with other Belgian and foreign data

Mean concentrations and profiles of PCBs of all individual and pooled serum samples were comparable with recent individual results obtained from Swedish (Grimvall et al., 1997) and Dutch women (Koopman-Esseboom et al., 1994a,b). In these studies the women were younger, namely in average 38.8 and 29 years old, respectively (Table 4). Regional differences for PCB concentrations between industrialised and rural areas were also investigated in the Netherlands (Koopman-Esseboom et al., 1994a). After the analysis of 406 individual maternal plasma and 172 individual human milk samples and covariate correction, they reported higher plasma levels of PCB 118 in the industrialised areas, but similar levels of PCB 138, 153 and 180. Furthermore, comparison is possible with two studies available in Belgium until now (Pauwels et al., 2000b; Nawrot et al., 2002). In the first study, PCBs were measured in young infertile women (mean age 32 years). The second study was part of the FLEHS, and was composed of 17–18 years old adolescents living in the two regions Antwerp and Peer. As expected, due to selection of elderly persons, concentrations of PCB 118, 138, 153 and 180 were higher in our population. Compared to the present study, a Canadian study (Longnecker et al., 2000) conducted in 1994 on 63 blood donors (33 females, 30 males) with mean age of 45 years showed approximately 3-fold lower levels of indicator, mono- and non-ortho PCBs. The burden of indicator PCBs was even 1.5–2-fold lower than presently found in young Flemish female populations (Pauwels et al., 2000b; Nawrot et al., 2002). This confirms that the PCB concentrations in Belgium and Europe remain a matter of concern.

The TEQ values in the serum of the Flemish women were higher than values found in Wallonia (Belgium) and other countries (Table 5). The data should be interpreted with caution due to differences in: sampling years, age of individuals and TEF values used (Safe, 1990; Ahlborg et al., 1994; Van den Berg et al., 1998). However, present levels in this Flemish age group can be compared with values obtained in other industrialised countries about 10 years ago. Moreover, a similar German population (43–71 years old) sampled in 1996 showed average values two times lower than PCDD/PCDF-TEQ values measured in the Flemish population (Table 5). As in the past, the PCDD/PCDF body burden values in the Flanders remains higher than in neighbouring countries. Regional differences of these compounds were not observed between pooled serum samples of both Flemish women groups. This could be eventually due to lack of power for the 47 pools (see above).

When including the non- and mono-ortho PCBs in the TEQ calculations, the total TEQ value increased with 33%. This was also observed in Canadian Red Cross blood donors from Toronto (Longnecker et al., 2000). In the latter study, both mean PCDD/PCDF-TEQs (20 pg TEQ/g fat) and total TEQs (35 pg TEQ/g fat) were lower than in the present study. It was one of the few studies where all dioxin-like compounds were measured in the same population. Another approach for assessing the total TEQ burden is the measurement of Ah-receptor activity of POPs in serum using a bioassay (in our case, the CALUX). CALUX-TEQ values reflect the toxicity of all POPs having a synergistic, additive and/or antagonistic interaction with the Ah-receptor. Therefore, the CALUX-TEQ values will differ from the chemically estimated TEQs, which are simply summated. In the present study, the absolute value of the CALUX-TEQ was somewhat lower than the PCDD/PCDF-TEQ, and about half of the total WHO-TEQ (detailed results are presented in Koppen et al., 2001). The observed CALUX-TEQ was comparable to the CALUX-TEQ of the young Flemish women (Pauwels et al., 2000a) and considerably lower than the CALUX-TEQ values (mean of 103.7 pg TEQ/g fat) measured in plasma of young Dutch women in 1990–1992 (Brouwer, 1997). This latter discrepancy could be due to different methodologies used. It was clear that this assay might offer new possibilities in monitoring TEQ values in human serum, but further interlaboratory validation of the absolute CALUX-TEQ values is necessary.

Table 4
Comparison of serum PCB concentrations (ng/g fat) in women living in Belgium and other European countries (mean and range or SD are given in subsequent columns)

Age women (years)	PCB 28	PCB 52	PCB 101	PCB 105	PCB 118	PCB 138	PCB 153	PCB 156	PCB 157	PCB 167	PCB 170	PCB 180
Belgium												
106 individual (Pauwels et al., 2000a)[a]												
31.9	–	–	–	–	27.7	69.9	94.5	–	–	–	na	72.0
24–42	–	–	–	–	16.2	30.7	45.2	–	–	–	na	35.7
47 pools (present study)[b]												
58.5	3.2	1.7	1.5	7.1[c]	29.2[c]	91.8[c]	167.6[c]	15.8[c]	2.4	4.4	40.1[c]	104.1[c]
50–65	nd(1)-30	nd(1)-13	nd(1)-7	4–17	17–74	50–179	104–316	9–30	nd(1)-6	nd(1)-19	31–72	66–192
200 individual (present study)[b]												
58.5	nd	nd	nd	–	–	116.9[c]	158.1[c]	–	–	–	–	115.4[c]
50–65	nd	nd	nd	–	–	30–305	53–447	–	–	–	–	40–334
120 individual (Nawrot et al., 2002)[b]												
17.4	nd	nd	nd	–	–	18.9[d]	32.9[d]	–	–	–	–	19.3[d]
17–18	nd	nd	nd	–	–	5–137	6–162	–	–	–	–	3–119
Sweden												
50 individual (Grimvall et al., 1997)[e]												
42.0	3.0	na	1.0	6.6	31.0	120.0	210.0	21.0	3.9	7.7	52.0	140.0
29–53	nd(0.8)-25	na	nd(0.7)-7	2–26	10–100	55–410	91–780	8–87	nd(1)-16	3–25	20–70	52–520
The Netherlands												
415 individual (Koopman Esseboom, 1994a,b)[f]												
29	–	–	–	–	32.0[g]	120.0[g]	182.0[g]	–	–	–	–	108.0[g]
–	–	–	–	–	4–120	26–320	36–500	–	–	–	–	16–620

na: not available, nd: not detectable.
[a] Sampling year: 1996–1998.
[b] Sampling year: 1999.
[c] Median.
[d] Geometric mean.
[e] Sampling year: 1986–1991.
[f] Sampling year: 1990–1992.
[g] Calculated from concentrations expressed in ng/g plasma assuming that 1 g = 1 ml plasma and a mean plasma fat concentration of 500 mg/dl.

Table 5
TEQ blood values from the CALUX-bioassay or chemical PCDD/PCDF analysis with GC–HRMS in some European studies

Country	Year	Age	Sex	*N* individual/ pool	Matrix	Mean pg TEQ/g fat	SD	Range	Analysis method	Reference
Belgium (Flanders)[a]	1999	58.5	Females	47 Pools	Serum	35.0	14.9	4.2–64.9	CALUX	Present study
				200 Individual		41.6[b]	–	2.1–139.6	CALUX	Present study
Belgium (Flanders)	1999	17–18	Males + females	200 Individual	Serum	30.9[b]	–	2.0–243.5	CALUX	Nawrot et al. (2002)
	1996–1998	32	Females	106 Individual		46.8 37.4[b]	43.8	2.0–160.2	CALUX	Pauwels et al. (2000a)
Netherlands	1990–1992	±30	Females	13 Individual	Plasma	103.7	51.5	–	CALUX	Brouwer (1997)
Belgium (Flanders)	1999	58.5	Females	47 Pools	Serum	48.0[bc]	–	31.2–81.3	GC–HRMS	Present study
Belgium (Wallonia)	1999	10–80	Males + females	54 Individual	Blood	36.7[d] (waste incinerators) 27.2[d] (controls)	–	–	GC–HRMS	Unpublished
Finland	1989–1990	41	Males	14 Individual	Blood	49[d]		20–99	GC–HRMS	EU Dioxin Exposure Data (1999)
	1993	43	Males	18 Individual		37[d]		26–86	GC–HRMS	EU Dioxin Exposure Data (1999)
Finland rural	1999	<30	Males			–		10–15	GC–HRMS	EU Dioxin Exposure Data (1999)
	1999	>30	Males			–		30–40	GC–HRMS	EU Dioxin Exposure Data (1999)
Germany	1988	–	Males + females	10 Individual	Blood	46.3[d]	–	–	GC–HRMS	EU Dioxin Exposure Data (1999)
	1989	37		102 Individual		40.8[d]	–	11.6–93.5	GC–HRMS	EU Dioxin Exposure Data (1999)
	1991	44.7		95 Individual		40.8[bd]	–	11.2–113.6	GC–HRMS	Ewers et al. (1996)
	1992	37		44 Individual		26.0[d]	–	12.0–61.0	GC–HRMS	EU Dioxin Exposure Data (1999)
	1993	37		70 Individual		21.7[d]	–	10.3-48.8	GC–HRMS	EU Dioxin Exposure Data (1999)
	1994	40.4		134 Individual		19.1[d]	–	5.2–43.9	GC–HRMS	EU Dioxin Exposure Data (1999)
	1996	36.7		180 Individual		16.5[d]	–	7.0–?	GC–HRMS	EU Dioxin Exposure Data (1999)
		18–30		59 Individual		13.0[d]	–	7.3–?		
		31–42		68 Individual		16.9[d]	–	7.0–?		
		43–71		53 Individual		19.9[d]	–	9.6–?		
Spain industrial	1997	28–62	Males + females	20 Individual	Plasma	27[d]	–	14.8–48.9	GC–HRMS	EU Dioxin Exposure Data (1999)

[a] Both analyses on same persons.
[b] Median.
[c] WHO-TEQ.
[d] I-TEQ.

Concentrations of most organochlorine pesticide in serum of the women were comparable to other studies. A recent study showed that plasma levels of PCP in men from Sweden and Latvia were between 170 and 1800 ng/g fat (Sjödin et al., 2000). The same range of PCP levels was found in blood from Canadian men and women participating in 1992 in the Santé Québec Health Survey and from a general population in Southern Quebec (Sandau et al., 2000). The levels of p,p'-DDE, HCB and γ-HCH observed in our study were similar or lower than levels from Great Lakes fish consumers (Anderson et al., 1998) and more than 10 times lower than plasma organochlorine levels in 65–74 years old German men and women, recruited in the early eighties (De Voto et al., 1998). In 1991–1992, p,p'-DDE was measured in old women, living in six European countries (Van't Veer et al., 1997). There, the average calculated blood concentration was about three times lower (2.5 ng/ml blood p,p'-DDE) than that observed in our study. Concentrations of p,p'-DDT were significantly higher in Peer. Both in Peer and Antwerp, the DDT/DDE ratio was very low (0.0036 and 0.0022 respectively), indicating a past exposure to p,p'-DDT. The use of p,p'-DDT in Belgium has been banned more than 25 years ago (1974). As the women have been living on average for almost 40 years in Peer and Antwerp, they might have been more exposed particularly in the rural area. This could also explain the higher (though not significant) average p,p'-DDE serum concentrations in Peer. A regional and historically explainable difference in p,p'-DDE concentrations was found in a study (Laden et al., 1999) nested in the US Nurses Health Study (1989–1990). It revealed significant higher plasma concentrations of p,p'-DDE in 30–50 years old women living in the Western United States (11 ng/ml), where p,p'-DDT was used more intensively in the past, compared to women from other parts of the country (6.3 ng/ml). The mean p,p'-DDE concentrations in our study (7.1 and 6.2 ng/ml p,p'-DDE in Peer and Antwerp, respectively) were of similar magnitude. Lindane concentrations were non-significantly higher in Peer ($p = 0.056$). Lindane remains in use in Belgium for restricted applications like disinfection of seeds, insecticide on soils, sugar beet, flowers and ornamental plants. Food is the main exposure route for the general population, so this might have smoothed the effect of lindane use in the rural area Peer. Dietary intake of fatty foods is also thought to be the main exposure way to HCB. However, the higher serum concentrations of HCB in Antwerp ($p = 0.001$) might suggest intake from industrial activities near to the city. HCB is a waste product in the production of several chlorinated hydrocarbons and some pesticides. It has been detected in treated waste-water from non-ferrous metal manufacturing, and is emitted in the atmosphere in flue gases and fly ash generated at waste incineration facilities (Oehme et al., 1987).

5. Conclusions

In the present study, concentrations of POPs (including organochlorine pesticides, PCBs, PCDD and PCDFs) were measured in serum of 50–65 years old Flemish women. The levels of indicator PCBs and organochlorine pesticides were comparable to those found in other European countries, while TEQ values were clearly higher. Based on pooled serum samples, concentrations of PCBs including indicator, mono-ortho and non-ortho PCBs were higher in the urban region compared to the more rural area. For these pooled samples total WHO-TEQ, PCDD/PCDF TEQ and CALUX-TEQ values were not different between the regions. Measuring TEQs in individual samples by the CALUX bioassay surprisingly revealed slightly higher values in the rural area. Because of statistical power loss and no possibility of confounder adjustment, pooling is generally not the most effective way to study regional differences. However, pooling of serum samples enabled to measure exposure in the whole study population on a more cost-effective way.

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References

Ahlborg, U.G., Becking, G.C., Birnbaum, L.S., Brouwer, A., Derks, H.J.G.M., Feeley, M.M., Golor, G., Hanberg, A., Larsen, J.C., Liem, A.K.D., Safe, S.H., Schlatter, C., Waern, F., Younes, M., Yrjanheikki, E., 1994. Toxic equivalency factors for dioxin-like PCBs. Chemosphere 28, 1049–1067.

Anderson, H.A., Falk, C., Hanrahan, L., Olson, J., Burse, V.W., Needham, L., Paschal, D., Patterson Jr., D., Hill Jr., R.H., 1998. Profiles of Great Lakes critical pollutants: a sentinel analysis of human blood and urine. Environ. Health Perspect. 106, 279–289.

Brouwer, A., 1997. Toetsing van het toxicologisch equivalente factoren (TEF) concept voor dioxinen en aanverwante stoffen met behulp van de CALUX-bioassay, Rapport CALUX-project.

Covaci, A., Schepens, P., 2001. Determination of selected POPs in human serum by solid phase disk extraction and GC–MS. Chemosphere 43, 439–447.

De Fré, R., Geuzens, R., Van Cleuvenbergen, R., Swaans, W., 1999. Dioxineconcentraties in depositie-en bodemstalen in de omgeving van Wilrijk. VITO report 1999/DIA/R/27.

De Voto, E., Kohlmeier, L., Heeschen, W., 1998. Some dietary predictors of plasma organochlorine concentrations in an

elderly German population. Arch. Environ. Health 53, 147–155.

EU Dioxin Exposure and Health Data, 1999. Report produced for European Commission Environment, UK Department of the Environment, Transport and the Regions (DETR). Available from http://europa.eu.int/comm/environment/dioxin.

Ewers, U., Wittsiepe, J., Schrey, P., Selenka, F., 1996. Levels of PCDD/PCDF in blood fat as indices of the PCDD/PCDF body burden in humans. Toxicol. Lett. 88, 327–334.

Fastabend, A., 2000. Determination of PCBs and organochlorine pesticides at environmental concentrations by GC-ECD using optimised procedures for sample preparation. Ph.D. Thesis. Universitat Chlausthal, Germany.

Grimvall, E., Rylander, L., Nilsson-Ehle, P., Nilsson, U., Stromberg, U., Hagmar, L., Ostman, C., 1997. Monitoring of PCBs in human blood plasma: methodological developments and influence of age, lactation and fish consumption. Arch. Environ. Contam. Toxicol. 32, 329–336.

Koopman-Esseboom, C., Huisman, M., Weisglas-Kuperus, N., Boersma, E.R., de Ridder, M.A.J., Van der Paauw, C.G., Tuinstra, L.G.M.Th., Sauer, P.J.J., 1994a. Dioxin and PCB levels in blood and human milk in relation to living areas in The Netherlands. Chemosphere 29, 2327–2338.

Koopman-Esseboom, C., Huisman, M., Weisglas-Kuperus, N., Van der Paauw, C.G., Tuinstra, L.G.M.Th., Boersma, E.R., Sauer, P.J.J., 1994b. PCB and dioxin levels in plasma and human milk of 418 Dutch women and their infants. Predictive value of PCB congener levels in maternal plasma for fetal and infant's exposure to PCBs and dioxins. Chemosphere 28, 1721–1732.

Koppen, G., Covaci, A., Van Cleuvenbergen, R., Schepens, P., Winneke, G., Nelen, V., Schoeters, G., 2001. Comparison of CALUX TEQ values with PCB and PCDD/F measurements in human serum of the Flanders Environmental and Health Study (FLEHS). Toxicol. Lett. 123 (1), 59–67.

Laden, F., Neas, L.M., Spiegelman, D., Hankinson, S.E., Willett, W.C., Ireland, K., Wolff, M.S., Hunter, D.J., 1999. Predictors of plasma concentrations of DDE and PCBs in a group of U.S. women. Environ. Health Perspect. 107, 75–81.

Longnecker, M.P., Ryan, J.J., Gladen, B.C., Schecter, A.J., 2000. Correlations among human plasma levels of dioxin-like compounds and polychlorinated biphenyls (PCBs) and implications for epidemiologic studies. Arch. Environ. Health 55, 195–200.

Murk, A.J., Leonards, P.E.G., van Hattum, B., Luit, R., van der Weiden, M.E.J., Smit, M., 1998. Application of biomarkers for exposure and effect of polyhalogenated aromatic hydrocarbons in naturally exposed European otters (*Lutra lutra*). Environ. Toxicol. Pharmacol. 6, 91–102.

Nawrot, T.S., Staessen, J.A., Den Hond, E.M., Koppen, G., Schoeters, G., Fagard, R., Thijs, L., Winneke, G., Roels, H.A., 2002. Determinants of PCBs and dioxin-like compounds in serum of adolescents. Environ. Health Perspect., submitted for publication.

Oehme, M., Mano, S., Mikalsen, A., 1987. Polyhalogenated and polycyclic compounds in the emissions of small and large-scale municipal waste incinerators. Chemosphere 16, 143–153.

Pauwels, A., Cenijn, P.H., Schepens, P.J.C., Brouwer, A., 2000a. Comparison of chemical-activated luciferase gene expression bioassay (CALUX) and gas chromatography for PCB determination in human serum and follicular fluid. Environ. Health Perspect. 108, 553–557.

Pauwels, A., Covaci, A., Weyler, J., Delbeke, L., Dhont, M., De Sutter, P., D'Hooghe, T., Schepens, P., 2000b. Comparison of persistent organic pollutant residues in serum and adipose tissue in a female population in Belgium, 1996–1998. Arch. Environ. Contam. Toxicol. 39, 265–270.

Safe, S., 1990. PCBs, PCDDs, PCDFs, and related compounds: environmental and mechanistic considerations which support the development of toxic equivalency factors (TEFs). Crit. Rev. Toxicol. 21, 51–88.

Sandau, C.D., Ayotte, P., Dewailly, E., Duffe, J., Norstrom, R.J., 2000. Analysis of hydroxylated metabolites of PCBs (OH-PCBs) and other chlorinated phenolic compounds in whole blood from Canadian Inuit. Environ. Health Perspect. 108, 611–616.

Schoeters, G., 1998. Inventarisatie en evaluatie van de milieuonderzoeken en medische onderzoeken in het kader van de ISVAG-huisvuilverbrandingsoven te Wilrijk. VITO report VITO/R/98.001.

Sjödin, A., Hagmar, L., Klasson-Wehler, E., Björk, J., Bergman, Å., 2000. Influence of the consumption of fatty Baltic sea fish on plasma levels of halogenated environmental contaminants in Latvian and Swedish men. Environ. Health Perspect. 108, 1035–1041.

Staessen, J.A., Roels, H.A., Den Hond, E., Schoeters, G., Koppen, G., Hoppenbrouwers, K., Nawrot, T., Nelen, V., Thijs, L., Vanderschueren, D., Van Hecke, E., Verschaeve, L., Vlietinck, R., Fagard, R., 2001. Renal function, cytogenetic measurements, and sexual development in adolescents in relation to environmental pollutants: a feasibility study of biomarkers. The Lancet 357, 1660–1669.

Tarkowski, S., Yrjänheikki, E., 1989. WHO-coordinated intercountry study on levels of PCDDs and PCDFs in human milk. Chemosphere 19, 553–557.

Van Cleuvenbergen, R., Wevers, M., Schoeters, G., De Fré, R., 1994. Dioxins in (PCDDs and PCDFs) in human milk from Flanders, Belgium: concentrations and congener profiles. Organohalogen Compd. 20, 215–220.

Van den Berg, M., Birnbaum, L.S., Bosveld, A.T.C., Brunstrom, B., Cook, P., Feeley, M., Giesy, J.P., Hanberg, A., Hasegawa, R., Kennedy, S.W., Kubiak, T.J., Larsen, J.C., van Leeuwen, R.F.X., Liem, A.K.D., Nolt, C., Peterson, R.E., Poellinger, L., Safe, S., Schrenk, D., Tillitt, D., Tysklind, M., Younes, M., Waern, F., Zacharewski, T., 1998. Toxic equivalency factors (TEFs) for PCBs, PCDDs and PCDFs for humans and wildlife. Environ. Health Perspect. 106, 775–792.

Van Erp-Baart, M.A. (Ed.), 1993. Nevo tabel, 1st ed. Den Haag.

Van Loon, H., Koppen, G., Van Larebeke N., Schoeters G., Nelen V., Staessen J., Roels H., Vlietinck B., 2002. The

flemish environment and health study (FLEHS): Area differences in exposure markers. Environ. Health Perspect., in press.

Van't Veer, P., Lobbezoo, I.E., Martín-Moreno, J.M., Guallar, E., Gómez-Aracena, J., Kardinaal, A.F.M., Kohlmeier, L., Martin, B.C., Strain, J.J., Thamm, M., van Zoonen, P., Baumann, B.A., Huttunen, J.K., Kok, F.J., 1997. DDT (dicophane) and postmenopausal breast cancer in Europe: a case-control study. Br. Med. J. 315, 81–85.

WHO, 1998. Consultation on assessment of the health risk of dioxins; re-evaluation of the tolerable daily intake (TDI). Food Addit. Contam. 17 (4), 223–240.